Correlation of striatal dopamine release and peripheral hypertension after transient ischemia in gerbils

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Purpose: **Intracranial norepinephrine release has been associated with post-carotid endarterectomy hypertension in human beings. To study this phenomenon under more controlled conditions, we studied the relationship of cerebral catecholamines and blood pressure in gerbils, whose cerebral circulation is similar to that in human beings.**
Methods: **Twelve anesthetized gerbils underwent iliac artery blood pressure monitoring and in vivo electrochemistry catecholamine monitoring with use of catecholamine-specific electrodes placed stereotactically into the cerebral striatum. Six gerbils underwent 10 minutes of bilateral carotid artery occlusion (ischemic), whereas six underwent carotid artery dissection without occlusion (control).**
Results: **The control group demonstrated a continuous gradual decline in blood pressure and striatal catecholamine during the 150-minute observation period. In contrast the ischemic gerbils demonstrated a sharp catecholamine rise during ischemia, a marked catecholamine drop shortly after carotid artery unclamping, and then a secondary larger catecholamine release that peaks in 60 minutes and gradually returns to baseline in 120 minutes. The blood pressure closely followed the catecholamine levels, with a sharp 20 mm Hg rise in blood pressure above baseline during carotid artery occlusion, followed by a dramatic 10 mm Hg drop below baseline after carotid artery unclamping and then a gradual rise of the blood pressure 25 mm Hg above baseline, which peaks in 80 minutes, with a gradual decline to the same blood pressure as in the control subjects 120 minutes after ischemia.**
Conclusion: **We conclude that striatal catecholamine release correlates with peripheral blood pressure during transient cerebral ischemia and reperfusion. This phenomenon may explain the mechanism of post-carotid endarterectomy hypertension in human beings, and this gerbil model can be used to study its prevention and treatment. (J VASC SURG 1995;22:135-41.)**

Postoperative hypertension lasting 12 to 24 hours complicates 19% to 66% of carotid endarterectomy cases. This transient hypertension is associated with a 10% to 47% incidence of postoperative neurologic deterioration.[1-6] In contrast, less than 6% of patients with hypertension or normal blood pressure have development of neurologic complications. Although a number of risk factors for the development of this complication have been identified, its mechanism still remains unclear.

Previously, we demonstrated a temporal correlation between post-carotid endarterectomy hypertension and elevated cranial norepinephrine levels in 47 patients who underwent unilateral or staged bilateral carotid endarterectomy.[7] Although the data suggested a central nervous system mechanism for the hypertension, too many variables could have influenced the development of the postoperative hypertension. Hence, we attempted to replicate these findings in controlled animal studies.

We chose the Mongolian gerbil as our experi-

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mental model for the following reasons. As is the case in human beings, 50% of these animals lack a complete circle of Willis, and unilateral carotid artery occlusion induces forebrain ischemia in approximately 50% of these animals.[8] Permanent bilateral carotid artery occlusion causes global forebrain ischemia in all animals,[9] and this ischemia produces a massive release of cerebral catecholamines.[9,10]

Our early experiments showed that 10 minutes of unilateral carotid artery clamping produced a biphasic catecholamine response that correlated with ischemia in approximately 50% of the gerbils (unpublished data, 1991). Furthermore, 10 minutes of bilateral carotid artery occlusion caused a uniformly reproducible biphasic release of the catecholamine dopamine in all animals: an initial transient increase during ischemia and a larger, more sustained catecholamine release during the reperfusion period.[11] A similar biphasic release of dopamine in the rat striatum was demonstrated subsequently by Wood et al.[12] The striatal dopamine release profile observed in gerbils and rats during the reperfusion period was similar to the norepinephrine profile we observed previously in human beings who had development of post-carotid endarterectomy hypertension.[7] Thus a possible temporal correlation between dopamine release in gerbil striatum and peripheral blood pressure elevation was postulated.

However, this correlation could not be determined because of the technical difficulty in obtaining blood pressure data in these extremely small (50 to 70 gm) and delicate animals. Existing tail blood pressure cuffs were too big and inaccurate. Further experimentation showed that aortic cannulation led to animal death, and femoral cannulation was technically too difficult (unpublished data, 1991). Recently we determined that iliac artery cannulation by a retroperitoneal approach was technically feasible and nonfatal (unpublished data, 1991). Hence, we now had a gerbil model that allowed simultaneous monitoring of striatal dopamine and peripheral blood pressure.

This model allowed us to test the hypothesis that elevated striatal dopamine during and after transient ischemia correlates with a postischemic hypertensive response and thus to provide inferential data to explain a possible mechanism for post-carotid endarterectomy hypertension. The testing of our hypothesis in this gerbil model is reported herein.

MATERIAL AND METHODS

Preparation of the male and female Mongolian gerbil (Tumblebrook Farms, West Brookfield, Mass.) and the chronoamperometric measurements of striatal dopamine release followed the protocol presented in our previous study[11] and will be briefly discussed. After maintenance of surgical anesthesia with 400 mg/kg choral hydrate administered intraperitoneally in gerbils weighing 50 to 70 gm, the common carotid arteries were exposed. Adjustable snares consisting of a strand of 5-0 silk suture and a small flexible tube were placed around each carotid artery to regulate blood flow.

Blood pressure was monitored from the right common iliac artery. A right oblique suprainguinal incision was made, and the right common and external iliac artery were dissected. The external iliac artery was incised transversely and cannulized with polyethylene 50 tubing. The tubing was advanced to the common iliac artery just below the aorta and secured with silk sutures. An online pressure transducer was connected to the tubing for continuous blood pressure measurement. Heparinized saline solution (100 units dissolved in 1000 ml 0.9% saline solution) was used to prevent thrombosis of the tubing.

Stearate-modified graphite paste working electrodes, which preferentially detect catecholamines,[13] were placed stereotactically in the right and left striatum (0.8 mm anterior and 2.8 mm lateral to the bregma, 3.6 mm below the dura, with the incisor bar set at −5 mm). The gerbil was placed in the prone position to allow electrode placement and accessibility of the carotid artery snares. A combined (silver/silver chloride[Ag/AgCl]) reference and stainless-steel auxiliary electrode was placed in fluid contact with the brain with normal saline solution. The core temperature for each gerbil was maintained at 37° C with self-regulating healing pads.

The electrodes were connected to an in-house built electrochemical device (Echempre/GMA Tech, Vancouver, British Columbia, Canada) that was capable of linear potential sweep voltammetry with semidifferential electroanalysis and repetitive chronoamperometry. Semiderivative voltammograms were obtained by use of a potential ramp from −200 to +350 mV at a scan rate of 10 mV/sec and a sampling interval of 10 minutes. After achieving a stable semiderivative voltammography baseline (approximately 90 minutes) with the semiderivative voltammography sampling mode, chronoamperometric measurements were taken. Chronoamperometric oxidation currents were measured at the end of a 1-second pulse that ranged from −100 to 250 mV (versus Ag/AgCl) with a 60-second sampling interval. After recording a stable chronoamperometric

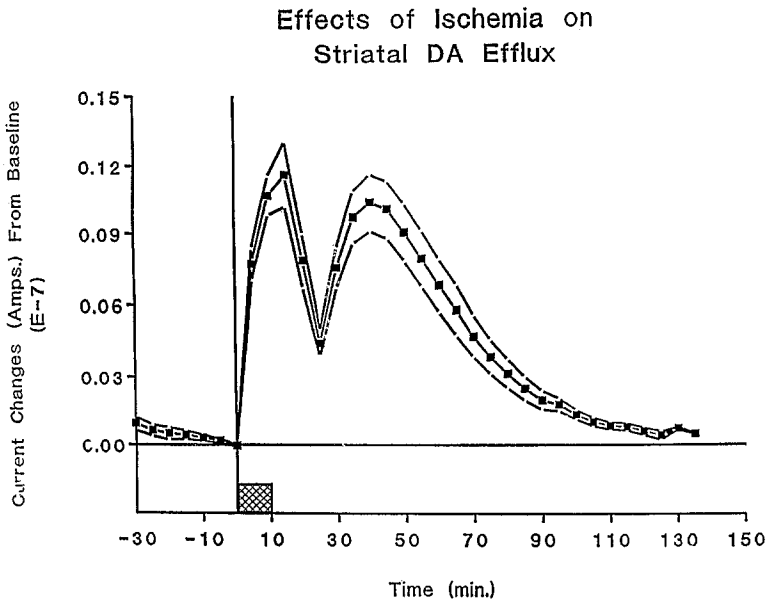

Fig. 1. Chronoamperometric recordings show effect of 10 minutes of bilateral carotid occlusion and subsequent reperfusion on caudate dopamine release in six gerbils. *Solid lines* represent standard deviation from mean.

baseline for 15 to 20 minutes, both carotid arteries were occluded with the adjustable snares for 10 minutes and then released. Chronoamperometric measurements were taken for at least 120 minutes after carotid artery clamping or until the signal returned to baseline levels.

Six gerbils underwent transient forebrain ischemia induced by 10 minutes of bilateral carotid artery occlusion. Six control animals underwent sham operations exactly as described above except that no transient ischemia was induced. At the end of the observation period, each animal was given an overdose of pentobarbital. Electrode placement in the striatum was verified by standard cresyl violet stain on frozen sections. The experiments of the control and ischemic animals were alternated to avoid any technical bias.

RESULTS

Fig.1 shows the biphasic efflux of striatal dopamine induced in six gerbils subjected to 10 minutes of ischemia. The first peak was 12 nA above baseline after occlusion and during the initial reperfusion period. As reperfusion progressed, the response rapidly fell to 5 nA above baseline approximately 25 minutes after the start of occlusion. Soon afterward the response rose to a second peak of 11 nA above baseline at 40 minutes after occlusion; the signal then returned to baseline over the next 80 minutes. The six control gerbils showed a stable chronoamperometric signal over the 120 minutes of observation (data not shown).

Fig. 2, *A* shows peripheral blood pressure change in ischemic and control animals. The control group of six gerbils demonstrated a gradual decline in blood pressure during the entire monitoring period. Quantitatively the blood pressure dropped 25 mm Hg within 120 minutes. The 10-minute ischemic group revealed a marked rise of 15 mm Hg in blood pressure after occlusion. A dramatic drop of 25 mm Hg in blood pressure occurred after reperfusion. As reperfusion continued, the blood pressure gradually rose again by 10 mm Hg and peaked at approximately 50 minutes after occlusion. The blood pressure gradually fell thereafter, returning to the same level as controls 130 minutes after occlusion.

Fig. 2, *B* shows the 10-minute ischemic blood pressure response normalized to the control group blood pressure response. With normalization to the continuous decline in blood pressure present in the control group, the initial rise during the ischemic period was approximately 20 mm Hg. The precipitous blood pressure fall during early reperfusion was approximately 25 mm Hg. However, the second rise in blood pressure climbed 25 mm Hg and peaked approximately 80 minutes after occlusion. Afterward, the blood pressure fell to the normalized baseline 130 minutes after occlusion.

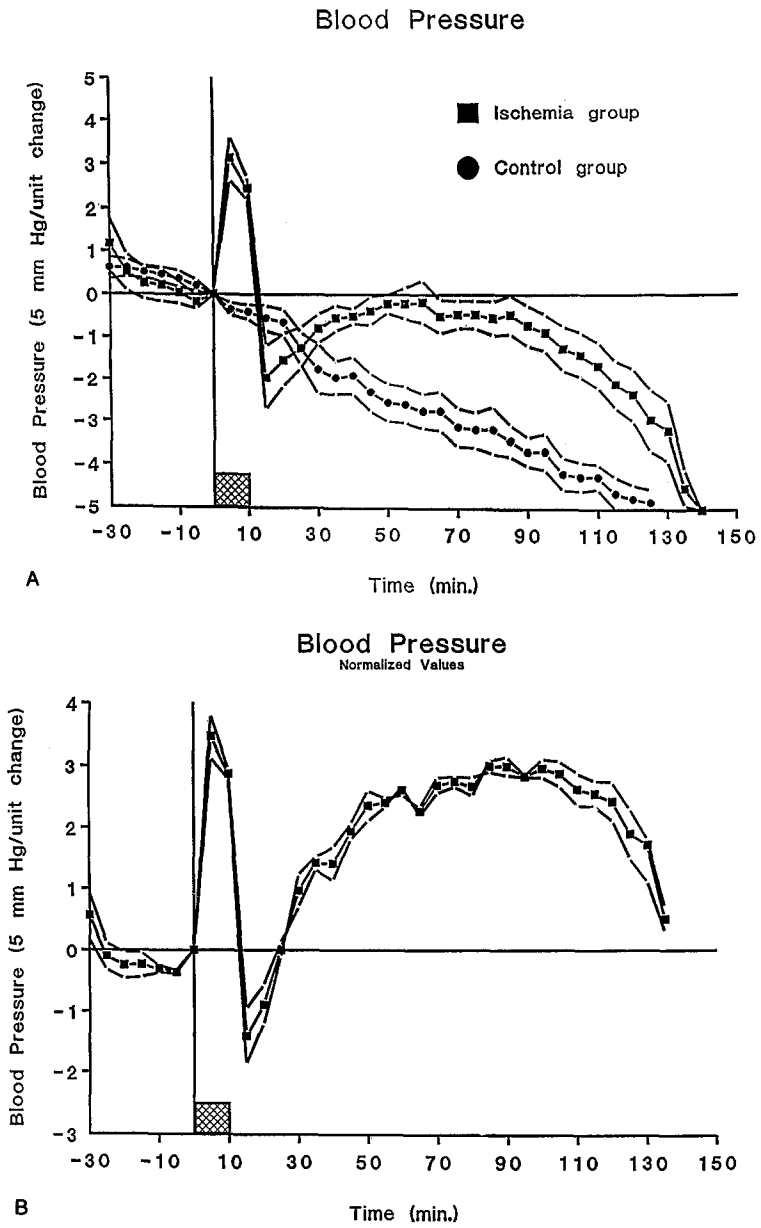

Fig. 2. A, Blood pressure values obtained from right common iliac artery of six gerbils subjected to 10 minutes of ischemia and six control animals. *Solid lines* represent standard deviation from mean. **B,** Normalized blood pressure values of ischemic versus control animals. Values were obtained by subtracting blood pressure of ischemic animals from control animals. *Solid lines* represent standard deviation from mean.

Fig. 3 shows the comparison of the striatal dopamine release to the blood pressure response and reveals a correlation between the biphasic changes in the two parameters. As dopamine concentration increased sharply during the ischemic period, the blood pressure also rose. Both responses decreased during the initial reperfusion and soon afterward rose to a second peak. Although dopamine declined to baseline levels thereafter, a sustained hypertensive state was observed at 50 minutes after occlusion and slowly returned to baseline over the next 80 minutes.

DISCUSSION

Postischemic hypertension induced in gerbils bears some similarities to the clinical picture of post-carotid endarterectomy hypertension. In our

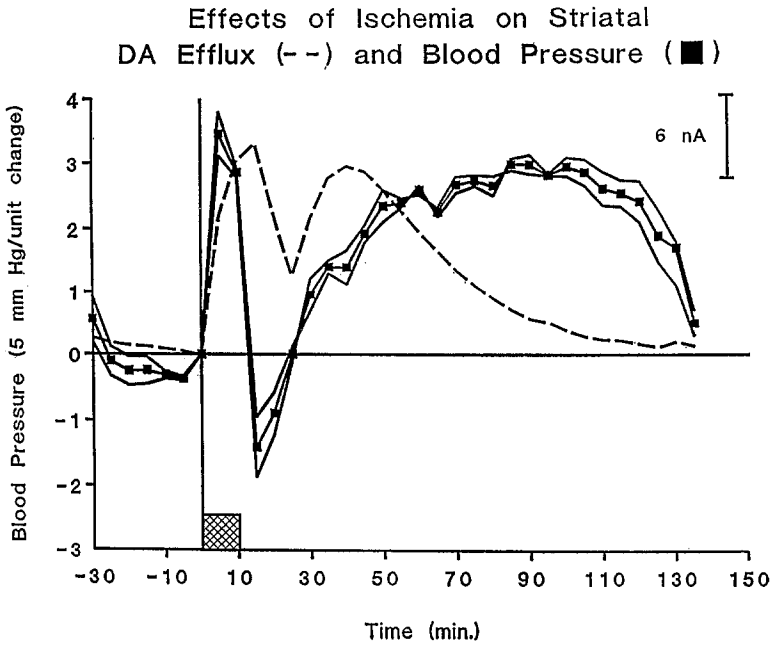

Fig. 3. Striatal dopamine efflux and normalized blood pressure values of six gerbils subjected to 10 minutes of bilateral carotid artery occlusion and six control animals. *Solid lines* represent standard deviation from mean.

previous study on human beings, we found a relationship between post-carotid endarterectomy hypertension and increases in cranial norepinephrine levels. In this animal study, striatal dopamine release and peripheral blood pressure changes also demonstrated a temporal correlation. During the ischemic period, both striatal dopamine efflux and peripheral blood pressure increased in time with respect to one another. Similarly, both responses decreased during the initial reperfusion period; but as reperfusion continued, a second peak was observed in both dopamine efflux and blood pressure. Thus these data support our previous clinical data that elevated catecholamine levels may play a part in the mechanism of postischemic hypertension.[7]

Although the findings in this gerbil study and the previous human study[7] are similar, some distinct differences are noteworthy. First of all, in the 47 patients who underwent carotid endarterectomy, dopamine, epinephrine, and norepinephrine were measured from the jugular vein; only norepinephrine increased significantly. In this gerbil study, only striatal dopamine levels were measured. Because norepinephrine concentrations are negligible in the striatum (being predominantly innervated by dopamine neurons), the actions of norepinephrine in other brain regions during ischemia remain unknown. However, catecholamines generally increase in a similar fashion in other brain regions.[14-17] In the previous clinical study,[7] central dopamine levels did increase, but this increase was not statistically significant, perhaps because dopamine levels measured from the jugular vein was diluted by the large cranial blood volume, and the striatum is proportionately much smaller in human beings than in gerbils.

Furthermore, modulation of blood pressure in human beings and gerbils may involve different catecholamines. Thus the particular catecholamine observed in the two studies may hold different relative importance in mediating blood pressure. For example, dopamine may mediate postischemic hypertension in gerbils, whereas post-carotid endarterectomy hypertension may be mediated predominantly by norepinephrine in human beings.[7] Although most studies have shown that the existence of a system in one animal also exists in another, a few recent studies have shown that different central nervous system mechanisms influence blood pressure control.[18,19] The importance of these different systems in terms of hypertension, or more specifically postischemic hypertension, has not been established yet.

Another difference in the two studies is that postischemic hypertension was induced in all animal cases as a matter of experimental design, whereas only 60% of the patients studied previously[7] had devel-

opment of postoperative hypertension. This discrepancy can be explained by the more advanced degree and duration of ischemia induced in the gerbil. In this experiment gerbils experienced complete forebrain ischemia from bilateral carotid artery occlusion, whereas the patients in the previous clinical study[7] underwent unilateral carotid artery occlusion with only a moderate degree of ischemia. Recently we demonstrated that the magnitude of striatal dopamine release is related to the duration and severity of the ischemic event.[11] Ten minutes of bilateral carotid artery clamping produced dopamine peaks 11 times that of 2 minutes of bilateral carotid artery clamping and 2 times that of 5 minutes of bilateral carotid artery clamping.[11] Furthermore, 10 minutes of unilateral carotid artery clamping produced catecholamine rises in only 50% of the gerbils (unpublished data). Thus we surmise that the gerbils, which underwent a greater and more consistent ischemic event than human beings, had development of a more consistent catecholamine release and hypertension.

The immediate rise in blood pressure on occlusion and the sharp drop on reperfusion occurred slightly earlier than changes in striatal dopamine efflux (Fig. 3). This early time-related discrepancy may be partially attributable to hemodynamic changes affecting the baroreceptors because the blood pressure changes were so immediate (within seconds). Occlusion of the carotid artery activates the baroreceptors, thereby inducing peripheral sympathetic activation to cause a rise in the blood pressure. In the immediate reperfusion period, the baroreceptors are depressed, thus inhibiting peripheral sympathetic activity with the resultant quick decrease in blood pressure.

However, these hemodynamic changes cannot adequately explain the subsequent delayed rise in blood pressure during late reperfusion with the correlated secondary rise in striatal dopamine release. Because the baroreceptors are depressed on reperfusion, peripheral blood pressure should decrease as a result of sympathetic inhibition and not increase as observed in this study. Thus postischemic hypertension exists beyond hemodynamic and baroreceptor influences and, instead, appears to be mediated by catecholamines.

The sustained hypertensive state observed in Fig. 3 may be interpreted in several ways. First of all, the striatal dopamine release observed in gerbils may not directly increase blood pressure, but work via another system that may use other catecholamines as their neurotransmitter, such as noradrenergic pathways in other parts of the brain, might induce the postischemic hypertension. In this study, only dopamine was measured because of the technical feasibility of reproducible probe placement in the relatively large striatum. However, several human studies show that other catecholamines correlate well with dopamine in response to ischemia and that norepinephrine in particular is the predominant catecholamine that affects blood pressure.[7,15,20,21] Another possibility is that acidic metabolites of dopamine that were not measured in this study, dihydroxyphenylacetic acid and homovanillic acid, might also be related to the hypertensive state. Last, postischemic hypertension may be caused by mechanisms still unknown. Hence, future studies to understand and modify this sustained hypertensive state are needed.

Much remains unknown about the gerbil animal model and warrants further investigation. For example, we do not know the normal blood pressure of gerbils, because so far we have been unable to find equipment to measure their blood pressures noninvasively. Furthermore, we do not know why the control gerbils show a gradual continuous 25 mm Hg drop of blood pressure over 130 minutes. A likely explanation is the prolonged anesthesia and volume depletion from the retroperitoneal exposure. However, the animal is too small and delicate to adequately assess or control these factors with our current techniques.

Nevertheless, the gerbil animal model described herein represents a significant progress in the study of post-carotid endarterectomy hypertension. We now have an animal model that allows future studies to be directed at manipulating this system with pharmacologic agents. Central alpha-antagonists such as clonidine and calcium channel blockers such as nimodipine are prime examples of antihypertensive drugs that could be tested to block this postischemic hypertensive response. Clonidine inhibits central noradrenergic sympathetic outflow and thereby reduces peripheral norepinephrine concentrations.[20,21] We previously observed that clonidine simultaneously reduced postoperative hypertension and intracranial norepinephrine in one patient who had post-carotid endarterectomy hypertension.[7] Nimodipine significantly reduces blood pressure in patients with mild and moderate essential hypertension by primarily limiting calcium ion entry through voltage-sensitive calcium channels at the cellular level in tissues of the central nervous system. Demonstration of these drugs blocking the catecholamine and hypertensive responses simultaneously would confirm our original hypothesis and provide important insight into the pathophysiologic study, treatment,

and prevention of post-carotid endarterectomy hypertension.

In conclusion, we have demonstrated that postischemic hypertension induced in gerbils resembles the clinical picture of post-carotid endarterectomy hypertension. Our gerbil model with simultaneous monitoring of intracranial dopamine and peripheral blood pressure values has shown that elevated dopamine levels after transient ischemia are temporally correlated with postischemic hypertension. Furthermore, this animal model offers the flexibility of controlled physiologic and pharmacologic manipulation. Future studies to further explain the mechanism of postischemic hypertension should be directed toward measuring different catecholamines in different areas of the brain and blocking the catecholamine and hypertensive responses pharmacologically with drugs such as clonidine and nimodipine.

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